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# Behavior of decabromodiphenyl ether (BDE-209) in soil: Effects of rhizosphere and mycorrhizal colonization of ryegrass roots

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*BDE-209 dissipation and degradation in soil were affected by both its proximity to ryegrass roots and inoculation with an AM fungus.*

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ABSTRACT

A rhizobox experiment was conducted to investigate degradation of decabromodiphenyl ether (BDE-209) in the rhizosphere of ryegrass and the influence of root colonization with an arbuscular mycorrhizal (AM) fungus. BDE-209 dissipation in soil varied with its proximity to the roots and was enhanced by AM inoculation. A negative correlation ($P < 0.001$, $R^2 = 0.66$) was found between the residual BDE-209 concentration in soil and soil microbial biomass estimated as the total phospholipid fatty acids, suggesting a contribution of microbial degradation to BDE-209 dissipation. Twelve and twenty-four lower brominated PBDEs were detected in soil and plant samples, respectively, with a higher proportion of di- through hepta-BDE congeners in the plant tissues than in the soils, indicating the occurrence of BDE-209 debromination in the soil-plant system. AM inoculation increased the levels of lower brominated PBDEs in ryegrass. These results provide important information about the behavior of BDE-209 in the soil-plant system.

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## 1. Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in plastics, textiles, and electronics (De Wit, 2002). Three PBDE technical mixtures were commercially available, that is, penta-BDE, octa-BDE, and deca-BDE. The former two products have been banned or voluntarily withdrawn from use in some regions of the world (Directive EEC, 2003; California State Assembly, 2003) and have also been included on the Stockholm Convention list of priority persistent organic pollutants. However, BDE-209 remains legal in most countries and is starting to be banned in some countries (Betts, 2008; BSEF, 2010). Owing to their high volume production, lipophilicity and persistence, PBDEs have become ubiquitous contaminants in the environment (Hites, 2004).

Soils represent a major sink for PBDEs released to the environment and reports have shown the ubiquitous distribution of PBDEs in soils (Hassanin et al., 2004; Zou et al., 2007; Hale et al., 2002). Degradation of PBDEs and particularly the highly brominated congeners in soil is an important process which determines their fate in the terrestrial environment. For example, BDE-209 is generally considered as having low toxicity, but evidence continues to mount that it can break down in the environment into other prohibited PBDEs with much higher toxicity (Betts, 2008). Plant-microbe interactions in soil have been reported to be a key factor influencing the degradation of halogenated organic contaminants (Vonderheide et al., 2006; Leigh et al., 2006; Xu et al., 2010). The rhizosphere comprises the zone of soil immediately surrounding plant roots and it has physical, chemical, and biological properties that are distinct from those of non-rhizosphere bulk soil (Phillips et al., 2003). Enhanced degradation in the rhizosphere is termed rhizodegradation (Stefan and Ulrich, 2001) and has been demonstrated for a range of organic pollutants including polycyclic aromatic hydrocarbons (PAHs) (Corgié et al., 2003), pentachlorophenol (He et al., 2005) and polychlorinated biphenyls (Ding et al., 2009). Previous studies have shown that plants can significantly enhance the degradation of PBDEs including BDE-209 in soils (Huang et al., 2010; Mueller et al., 2006). Nevertheless, rhizosphere effects on PBDE degradation have not been elucidated. Furthermore, the rhizosphere represents a highly dynamic environment involving interactions between competing roots and pathogenic/nonpathogenic microbes and soil invertebrates (Hirsch et al., 2003). Therefore, rhizodegradation might show a distance-dependent pattern of behavior. However, few studies have considered the distance-dependent aspects of rhizodegradation (He et al., 2005; Joner and Leyval, 2003; Corgié et al., 2003).

Arbuscular mycorrhiza (AM) is a ubiquitous association between soil-borne fungi and the roots of most terrestrial plant species

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(Smith and Read, 1997), and mycorrhizal colonization can result in quantitative and qualitative changes in root exudation and in the soil microbial community, particularly in the rhizosphere. We therefore hypothesized that AM fungi may influence the degradation of PBDEs in soils, particularly in the rhizosphere. Evidence has been reported on enhanced dissipation of PAHs in soils (Joner and Leyval, 2003). However, we are only starting to learn about the effects of AM fungi on degradation of organic pollutants and whether or not they influence the degradation of PBDEs in soil remains unknown.

In the present study, which was part of a wider investigation into the fate of BDE-209 in soil (Huang et al., 2010), a greenhouse rhizobox experiment was conducted to examine its degradation and dissipation in response to its proximity to ryegrass roots in order to elucidate rhizosphere effects on PBDE degradation. Debrominated products of BDE-209 in plants and soils were identified. The effect of the AM fungus *Glomus mosseae* on the degradation of BDE-209 and on its accumulation and metabolism in plants were also examined to elucidate the importance of mycorrhizal colonization and the rhizosphere effect in the degradation of BDE-209 in soil.

## 2. Materials and methods

### 2.1. Chemicals

Standards of BDE-209, PCB-30, PCB-209 and BDE-77 were obtained from Sigma–Aldrich (Sigma–Aldrich, Inc., St. Louis, MO, USA) and a standard solution of PBDEs containing 27 native congeners was purchased from Wellington Laboratories, Inc., Guelph, Ontario, Canada. Details of the congeners are supplied in Table S1 of the Supplementary material. All solvents used, i.e. *n*-hexane, dichloromethane, toluene, chloroform, acetone and methanol, were of HPLC grade. Distilled water was used in all of the experiments. Anhydrous sodium sulphate ($Na_2SO_4$), silica gel and alumina (100–200 mesh) were washed with hexane and used after heating overnight at 150 °C.

### 2.2. Experimental design

The rhizobox design was similar to that described by He et al. (2005), with dimensions of 110 mm × 80 mm × 120 mm (length × width × height). Each rhizobox was divided into three sections: a central compartment for root growth (30 mm in width), five sandwiching layers (2 mm each) on both sides next to the root compartment separated by nylon mesh (37 μm) representing rhizospere zones, and an outer compartment (>10 mm) on both sides representing the outer rhizosphere zone. The nylon mesh was used to separate the compartments and permit water, nutrients and root exudates as well as soil microorganisms to move freely between layers, but restrict root entry.

A loamy soil without detectable PBDEs was used in this experiment. Its selected characteristics are as follows: pH (in water) 7.75, organic matter 2.5%, cation exchange capacity 25.8 cmol $kg^{-1}$, $NaHCO_3$-extractable P 4.5 mg $kg^{-1}$, clay 20%, silt 35% and sand 32%. Methods for soil treatment, spiking of BDE-209 and incubation are provided in the Supplementary material. The final concentration was $3584.0 \pm 201.6$ ng $g^{-1}$ after incubation for 4 months prior to plant cultivation. BDE-206, -207, -208, were detected at concentrations of $28.7 \pm 1.5$, $37.6 \pm 2.9$, and $17.8 \pm 1.1$ ng $g^{-1}$, respectively, all together a concentration of less than 2.3% of BDE-209. No other PBDEs were detected in the soil.

Inoculum of the AM fungus (*Glomus mosseae*, BGC GD01A) comprising a sandy soil containing spores, mycelium and broomcorn root fragments, was air-dried and sieved (2 mm). Italian ryegrass (*Lolium multiflorum* L.) seeds were purchased from the Chinese Academy of Agricultural Sciences, Beijing. They were surface sterilized in a 10% $H_2O_2$ (V/V) solution for 15 min, rinsed with sterile distilled water and pre-germinated on moist filter paper in the dark.

Each rhizobox received 1100 g of soil and was equilibrated in a growth chamber for 3 days at 60% of water holding capacity. Mycorrhizal treatments received 30 g of the fungal inoculum by mixing with about 280 g of soil and then placing the mixture in the root compartment of the rhizobox. Each rhizobox contained about 1500 spores. The non-mycorrhizal rhizoboxes were set up using an equivalent amount of sterilized inoculum together with an aqueous filtrate (sieving through a 20 μm nylon mesh) of non-sterilized soil to provide a similar microflora except for the absence of the AM fungus. The upper 0.5–1.0 cm of each pot was covered with non-spiked sterilized soil to establish a buffer layer to minimize the loss of BDE-209 due to evaporation and photolysis. Twelve pre-germinated ryegrass seeds were sown in each root compartment and thinned to eight seedlings after growth for one week. The pots were positioned randomly and re-randomized every 3 days. The experiment was conducted in a controlled-environment growth chamber with a photoperiod of 14 h at a light intensity of 250 μmol $m^{-2}s^{-1}$ provided by supplementary illumination. The day/night temperature regime was 25 °C/20 °C and the relative humidity was maintained at 70%. Distilled water was added as required to maintain soil moisture content at about 70% of water holding capacity by regular weighing. Non-spiked soil with plant growth and spiked soil without plant growth were included as controls and all the treatments were set up in triplicate.

### 2.3. Sample preparation

Plant shoots and roots were harvested separately after growth for 60 d. Root fragments were collected by sieving the soil and adding them to the root samples. The BDE-209 free soil in the upper layer was removed and then the bulk soil in each compartment was collected separately. Root samples were first carefully washed with tap water to remove any adhering soil particles. Then shoot and root samples were rinsed thoroughly with distilled water, blotted with tissue paper and weighed. A portion of fresh root sub-sample was taken from each treatment for the determination of AM colonization. Other root and shoot materials were then frozen at −50 °C overnight, freeze-dried for 48 h in a lyophilizer (FD-1, Beijing Boyikang Instrument Ltd, Beijing, China), and weighed to determine their dry weights. The dried root and shoot samples were then ground separately and stored in glass containers at −20 °C before chemical analysis. The soil samples for the analysis of PBDEs and phospholipid fatty acid (PLFA) profiling were freeze-dried and stored at −20 °C until use.

### 2.4. Chemical extraction and analysis

AM colonization was assessed following the same principles as employed previously in our laboratory (Wu et al., 2008a). PLFA extraction and analysis were conducted according to the method of Frostegård et al. (1993). Detailed procedures are provided in the Supplementary material. Fatty acids were analyzed on an Agilent 7890 GC-MS (5975 inert) (Agilent, Palo Alto, CA, USA).

Extraction and cleanup of PBDEs in soil and plant samples were based on the method reported by Mai et al. (2005). PCB-30 and PCB-209 were added as surrogate standards to the samples prior to extraction and BDE-77 was added to the final solutions as an internal standard. An Agilent 6890II gas chromatograph (GC) equipped with a μ-electron capture detector (GC-μECD) was used for PBDE analysis. A DB-5 HT column (15 m × 0.25 mm × 0.10 μm) was used for the determination of BDE-209 and an HP-5MS column (30 m × 0.32 mm × 0.25 μm) for the lower brominated PBDEs. Detailed procedures are provided in the Supplementary material.

### 2.5. Quality assurance and quality control

Quality assurance protocol included the addition of surrogate standards and random injection of solvent blanks and standards. The limits of detection (LOD), three times the single-to-noise ratio, were in the range of 6–2000 pg $g^{-1}$ for all the PBDE congeners ($n = 6$), and details are provided in the Supplementary material (Table S1). Recoveries of the surrogate standards of PCB-30 and PCB-209 were 72.1–85.3% and 82.0–96.2%, respectively. Recoveries of the 28 congeners ranged from 70.8 to 111.2% with relative standard deviations (RSD) < 10% in spiked blank samples and from 65.8 to 117.2% with RSD < 15% in matrix spiked samples. No PBDE was found in the blank soil.

### 2.6. Data analysis

The data were subjected to statistical analysis by using the SPSS version 11.5 software package. Means and standard deviation were calculated for triplicates. Analysis of variance (ANOVA) was used to examine the significance of BDE-209 accumulation in plants and dissipation in soil as affected by AM inoculation or by the proximity to the roots.

## 3. Results and discussion

### 3.1. BDE-209 dissipation in soil

The residual concentrations of BDE-209 in the soil are plotted against the distance from roots (Fig. 1). BDE-209 dissipation in soil was detected after plant harvest and it was affected by the proximity to the roots. The residual BDE-209 concentration was lowest in the root compartment, significantly increased with increasing distance from the roots until a distance of 4 mm from the root compartment ($P < 0.05$), and from 4 mm outwards the differences were not significant from each other ($P > 0.05$). Concentrations of BDE-209 in the soils decreased by 20.8–56.4% and 15.3–45.3% compared with the initial concentrations in inoculated and uninoculated treatments, respectively. The change in residual BDE-209 concentration in the soils in response to the proximity to the roots showed similar

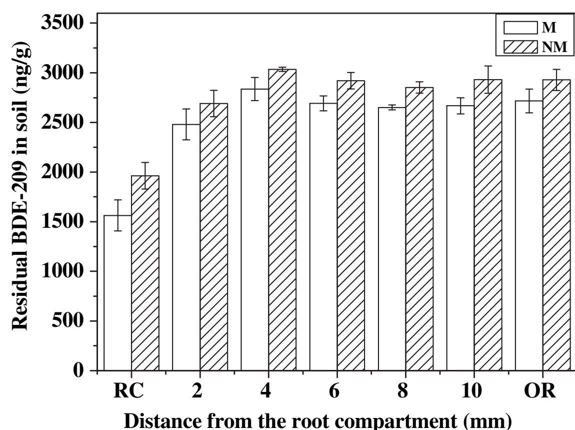


**Fig. 1.** Residual BDE-209 concentrations in the soil as a function of proximity to ryegrass roots. (Mycorrhizal (M); Non-mycorrhizal (NM); Root compartment (RC); Outer rhizosphere (OR)). Error bars denote standard error of the mean.

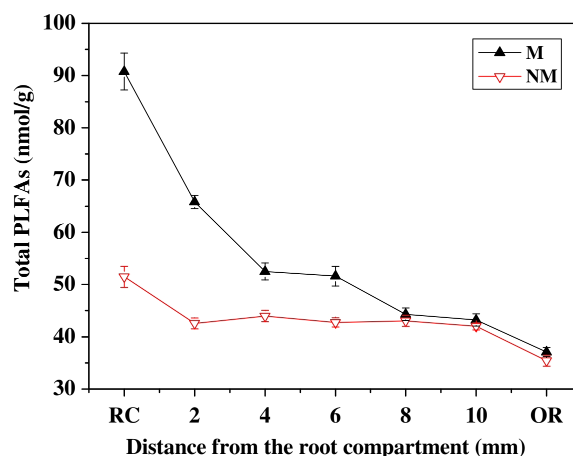


**Fig. 2.** Total phospholipid fatty acids (PLFAs) in the soil as a function of proximity to ryegrass roots. Error bars denote standard error of the mean.

trends in inoculated and uninoculated treatments. However, the residual BDE-209 concentrations in the soils were consistently lower in inoculated than in uninoculated rhizoboxes with 20.4% in the root compartment and 6.5–9.0% in the rhizospere zones, respectively. This observation is similar to that by Joner and Leyval (2003) for dissipation of PAHs in soils and provides further evidence for the beneficial effects of AM fungi on degradation of soil organic contaminants, even those with relatively high hydrophobicity.

Dissipation of BDE-209 in the soil can be ascribed largely to the contribution from its degradation, plant uptake and losses due to volatilization and sorption on either the rhizobox or the nylon meshes. The BDE-209 concentration in the unplanted control soil at the end of the pot experiment was 3470.0 ± 185.5 ng $g^{-1}$ on average, showing a decrease of only about 3% compared with the initial concentration of 3584.0 ± 201.6 ng $g^{-1}$. The total amount of BDE-209 lost by adsorption on nylon meshes and rhizobox was about 0.06% of BDE-209 in the soil. Plant uptake contributed 0.35–0.38% of the loss of BDE-209 in the soil. The sum of these losses cannot account for the total decrease in BDE-209 concentration in the soil, therefore degradation was speculated to be the major contributor to BDE-209 dissipation in soil.

Soil microbes are important for metabolism and degradation of halogenated organic contaminants in soils (Field and Sierra-Alvarez, 2008; Huang et al., 2011; Wu et al., 2008b) and likely play a key role in the degradation of BDE-209. PLFA analysis is a valuable method for tracking soil microbial profiles and determining soil microbial biomass (Hill et al., 2000; Joner et al., 2001). Therefore, PLFAs in the soils were analyzed and a total of 29 PLFAs were identified. The dynamics of the total PLFAs in soils were affected by the proximity to the roots (Fig. 2) and showed an approximately opposite trend to the changes in residual concentration of BDE-209 in the soil. Mycorrhizal inoculation increased the total PLFAs, particularly in the soils in and close to the root compartment. A negative correlation was found between the residual concentration of BDE-209 and the total PLFAs in the soil (Fig. 3, $P < 0.001$, $R^2 = 0.66$), supporting the above hypothesis that microbial metabolism and degradation contribute greatly to the dissipation of BDE-209. The modified conditions for microbial growth in soil as affected by plant growth and mycorrhizal inoculation increase the microbial biomass and also change microbial community composition. Data analysis shows a significant positive correlation between BDE-209 dissipation rate in soil and some specific PLFAs including the signature fatty acid of AM fungi (16:1ω5t; $P < 0.001$, $R^2 = 0.75$), markers of bacteria of 16:0 ($P < 0.01$, $R^2 = 0.63$), 18:1ω7c ($P < 0.001$, $R^2 = 0.70$), 18:1ω7t ($P < 0.01$, $R^2 = 0.64$) and cy24:0 ($P < 0.01$, $R^2 = 0.50$), indicating the potentially important role of the AM fungi and these soil bacteria in the degradation of BDE-209.

### 3.2. BDE-209 debromination in soil

A total of 12 lower brominated PBDEs (di- through nona-) were detected in the soil after ryegrass cultivation. Mean concentrations of individual lower brominated PBDE congeners in the soils are drawn as a function of proximity to ryegrass roots in Fig. 4. For the ranges of replicates, see Table S2 of the Supplementary material. Nona-BDE -206, -207, and -208 were the main debromination products and the other congeners were present at relatively low concentrations because BDE-209 is more likely to lose one bromine atom to form nona-BDE. In the absence of inoculation the total concentration of the lower debrominated PBDE congeners in the soil showed a decreasing trend from the roots to a distance of 6 mm from the root compartment and then increasing outwards. However, an almost even distribution of the total concentrations in the soil with increasing distance was observed in inoculated rhizoboxes until the outer compartment where the concentration increased. It is difficult to elucidate accurately the response of debromination of BDE-209 in soil to its proximity since the lower debrominated PBDE congeners determined in the soils represent

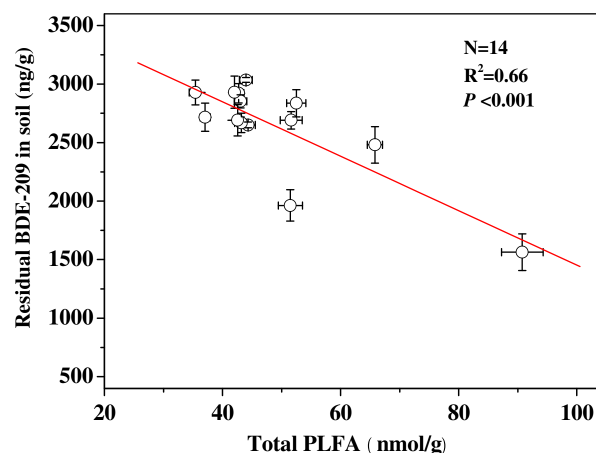


**Fig. 3.** Relationship between residual concentrations of BDE-209 and the total PLFAs in the soil. Error bars denote standard error of the mean.

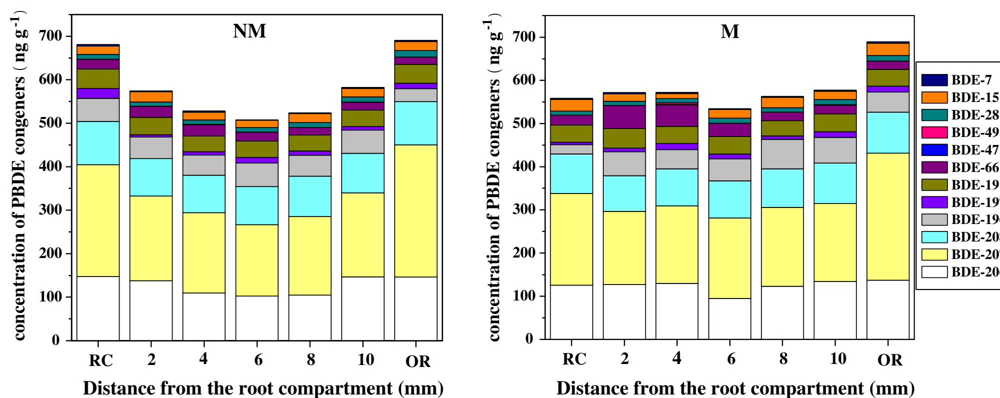


**Fig. 4.** Concentrations of di- through nona- brominated congeners in the soil as a function of proximity to ryegrass roots for non-mycorrhizal and mycorrhizal treatments.

the contributions of debromination, plant uptake and transportation of both BDE-209 and the debromination products. The U-type distribution of the lower brominated PBDEs in the absence of inoculation might be due to a high degradation rate of BDE-209 in the root compartment (see data in Table S2) and limitation in transportation and plant uptake of the lower brominated PBDEs in the outer compartment, leading to a higher concentration of PBDEs in the soils in the root and outer compartments. The almost even distribution of PBDEs in inoculated rhizoboxes might be ascribed to the enhanced transportation of the lower brominated PBDEs in the soil as the result of mycorrhizal inoculation increasing root exudates or soil microbes, and a hyphal effect (Wu et al., 2008b; Joner and Leyval, 2003). However, these explanations are speculative and require further investigation.

### *3.3. Accumulation and metabolism of BDE-209 in ryegrass*

Mycorrhizal colonization was observed only in AM-inoculated treatments at rates of $38.9 \pm 0.05\%$ and $16.8 \pm 0.02\%$ for non-spiked and spiked soils respectively. Both application of BDE-209 and colonization did not significantly affect the dry weights of shoots and roots (Table S3 in the Supplementary material, $P > 0.05$).

To acquire a further understanding of the influence of mycorrhizal inoculation on the behavior of BDE-209 in the soil-plant system, plant accumulation of BDE-209 was further determined. Shoot concentrations of BDE-209 in plants growing in non-spiked soil showed an average concentration of $1.90 \pm 0.03$ ng $g^{-1}$, less than 4% of the concentration in the plants growing in BDE-209 spiked soil. This suggests insignificant foliar uptake from the air based on the surface layer of non-spiked sterilized soil used to prevent BDE-209 evaporation from the soil in this experiment. Accumulation of BDE-209 in ryegrass was found in both roots and shoots and the mean concentration was much higher in roots than in shoots (Table 1). This evidence suggests that accumulation of BDE-209 in shoots may be attributed to root uptake and subsequent translocation within the plants. Concentrations of BDE-209 were higher in mycorrhizal roots than in non-mycorrhizal controls (11.2%, $P < 0.05$), suggesting the importance of inoculation on root uptake and accumulation of BDE-209. However, no significant difference in BDE-209 concentration in shoots existed between mycorrhizal and non-mycorrhizal plants.

A total of 24 lower brominated PBDEs (di- through nona-) were detected in the plant samples (Table 1 as bromine number and Table S4 in the Supplementary material for each congener). The total amount of lower brominated PBDEs was higher in roots than in shoots. Nona- and octa-BDEs, the more highly brominated congeners, were higher in roots than in shoots, but it is difficult to find a general pattern for other congeners in roots and shoots. Furthermore, more kinds of the lower brominated congeners were detected in the plant tissues (Table S4) than in the soils (Table S2), and the distribution pattern of lower brominated PBDEs in plant tissues was different from that in the soil, with a relatively higher proportion of di- through hepta-BDEs in plant tissues than in the soils (Fig. 5). These findings suggest the occurrence of debromination of BDE-209 in the soil and further debromination of the lower debrominated PBDEs within the plants. AM inoculation increased the total amounts of the debrominated products in ryegrass, especially in the shoots (Table 1). However, it is difficult to determine whether this effect of inoculation resulted from enhanced debromination inside the plants or from increased translocation of lower brominated PBDEs.

**Table 1**
Concentrations of PBDEs in ryegrass roots and shoots on dry weight basis (nmol $g^{-1}$)

| Brominated homologues | Roots | | Shoots | |
|---|---|---|---|---|
| | NM | M | NM | M |
| Di-BDE | 0.04 (0.01) | 0.03 (0.01) | 0.01 (0.001) | 0.04 (0.003) |
| Tri-BDE | 0.03 (0.002) | 0.04 (0.01) | 0.02 (0.001) | 0.02 (0.001) |
| Tetra-BDE | 0.06 (0.004) | 0.03 (0.002) | 0.03 (0.002) | 0.07 (0.01) |
| Penta-BDE | n.d. | 0.05 (0.01) | 0.01 (0.002) | 0.03 (0.003) |
| Hexa-BDE | 0.08 (0.004) | 0.15 (0.02) | 0.22 (0.01) | 0.39 (0.02) |
| Hepta-BDE | 0.05 (0.003) | 0.17 (0.03) | 0.11 (0.02) | 0.15 (0.01) |
| Octa-BDE | 0.12 (0.01) | 0.12 (0.02) | 0.03 (0.002) | 0.06 (0.004) |
| Nona-BDE | 0.33 (0.01) | 0.33 (0.04) | 0.11(0.02) | 0.12 (0.01) |
| Di- through nona-BDEs | 0.71 (0.02) | 0.92 (0.03) | 0.54 (0.02) | 0.88 (0.03) |
| BDE-209 | 1.52 (0.07) | 1.70 (0.07) | 0.05 (0.002) | 0.06 (0.002) |

Data within parenthesis are standard errors.

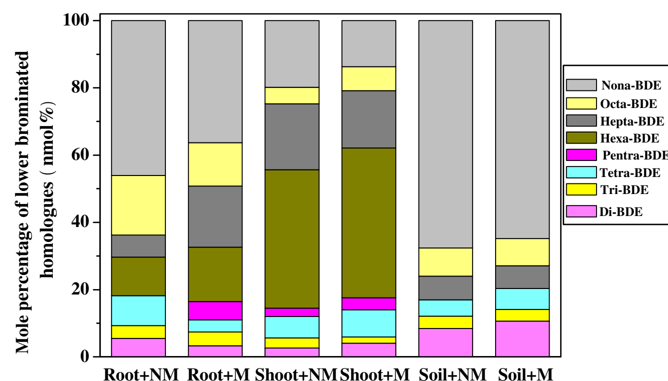


**Fig. 5.** Mole percentage of lower brominated homologues in the soil and ryegrass plants. The results for the soil represent for the whole soil of root and rhizosphere compartments.

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## Appendix. Supplementary material

Supplementary material associated with this paper can be found, in the online version, at doi:10.1016/j.envpol.2010.11.035.

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